Translation and processing of normal (PiMM) and abnormal (PiZZ) human α_1-antitrypsin

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Human liver mRNA isolated from subjects phenotyped as homozygous PiMM or PiZZ α_1-antitrypsin, was translated in a reticulocyte cell-free system, and α_1-antitrypsin identified by immunoprecipitation. In the presence of dog pancreas membranes the translated α_1-antitrypsin appeared as a larger product. Treatment with endo-β-*N*-glucosaminidase yielded a protein smaller than the reticulocyte translated product, presumably due to removal of the N-terminal signal sequence by membranes and sugar residues by endo-β-*N*-glucosaminidase. Quantitation of α_1-antitrypsin translated from PiMM and PiZZ livers suggests that both mRNA species were present at the same cellular concentration, and that processing to the core glycosylation stage proceeded at identical rates.

α_1-Antitrypsin *In vitro translation* *In vitro processing* *Human liver mRNA*

1. INTRODUCTION

α_1-Antitrypsin is a glycoprotein of M_r 51000 which functions as the major serine proteinase inhibitor in plasma. The structure, function and common abnormalities have been recently reviewed [1]. The common Z variant results in a plasma concentration in the PiZZ homozygote of about 15% of the PiMM normal. Studies measuring the half-life of circulating Z protein have excluded an increased rate of degradation [2] and it is likely therefore that the low plasma concentration results from either a decreased level of synthesis or from faulty processing. In this study, using cell-free systems, we have shown that the rates of translation of M and Z mRNA and of translocation and core glycosylation of the two polypeptides are equivalent.

2. MATERIALS AND METHODS

L-[^{35}S]methionine (700–1300 Ci/mmol) was obtained from Amersham; Oligo(dT)-cellulose (type 2) from Collaborative Research; Enhance and ^{14}C-labelled M_r standards from New England Nuclear. All other enzymes and chemicals were obtained from sources already described [3,4].

Human liver was obtained as in [5,6] and stored at −80°C until used. The RNA was isolated by the direct phenol method [7,8] and the poly(A)-containing RNA species purified by binding twice to oligo(dT)-cellulose at 400 mM NaCl without prior removal of DNA [6]. In vitro translation of mRNA was performed in a nuclease-treated rabbit reticulocyte lysate [9,10]. Incubations were for 90 min at 35°C and in some experiments nuclease-treated dog pancreas membranes [11] were present at a final concentration of 2.3 A_{260} units/ml. α_1-Antitrypsin was immunoprecipitated from translation assays as in [12] using monospecific antisera prepared in rabbits. Antibody precipitated translation products were analysed on SDS–polyacrylamide gel slabs using fluorographic enhancement [13]. Endo-β-*N*-glucosaminidase was purchased from Miles Laboratories, USA. Digestion was carried out as in [13] in the presence of PMSF as a protease inhibitor.

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3. RESULTS AND DISCUSSION

Human liver mRNA was isolated in yields varying from 25 μg to 60 μg mRNA/g wet wt tissue (1 A_{260} = 40 μg). Reticulocyte lysate cell-free synthesis was optimised for the translation of human liver mRNA. Maximum stimulation was achieved at a final [K^+] 110 mM with [Mg^{2+}] 1.65 mM. The addition of human placenta ribonuclease inhibitor [14] had no effect on either the stimulation of total protein synthesis or on the SDS–polyacrylamide gel profile.

When mRNA from both the PiMM and PiZZ phenotype livers was translated in the reticulocyte lysate system essentially identical stimulation was achieved (fig.1A). Maximum stimulation (4–10 × background) occurred at an mRNA concentration of 6.25 μg/assay. The same mRNA concentration gave maximum stimulation in the presence of dog pancreas membranes, although in some cases the membrane preparations inhibited total protein synthesis by up to 40%. α_1-Antitrypsin production was assayed by antibody precipitation (fig.1B) and again maximum stimulation occurred at an mRNA concentration of 6.25 μg/assay. Yields varied from 1.5–3.5% of total protein synthesised. No difference was noted in the amount of α_1-antitrypsin synthesised in the presence of membranes, and no selective inhibition of either the M or Z α_1-antitrypsin directed protein synthesis was observed.

SDS–polyacrylamide gel electrophoresis was performed on the antibody precipitates to confirm that the α_1-antitrypsin had undergone initial secretory processing by the membranes. Fig.2 (lanes 2 and 3) shows the profile of PiMM and PiZZ α_1-antitrypsin translated in the reticulocyte lysate. The two tracks are identical with the major band having an app. M_r 49000 and a second smaller product M_r 46000. These bands were both confirmed as α_1-antitrypsin by adding purified human α_1-antitrypsin to the translation mix prior to antibody precipitation. Both bands disappeared from the gel, indicating that both proteins were recognised by the α_1-antitrypsin antibody. A possible explanation for the second, lower M_r band is thiol-protease activity in the reticulocyte lysate which is known to cleave α_1-antitrypsin [15,16]. The gel profile of PiMM and PiZZ mRNA translated in the presence of membranes showed a single band at M_r 59000 (fig.2, lanes 4 and 5). When the cell-free assays performed in the

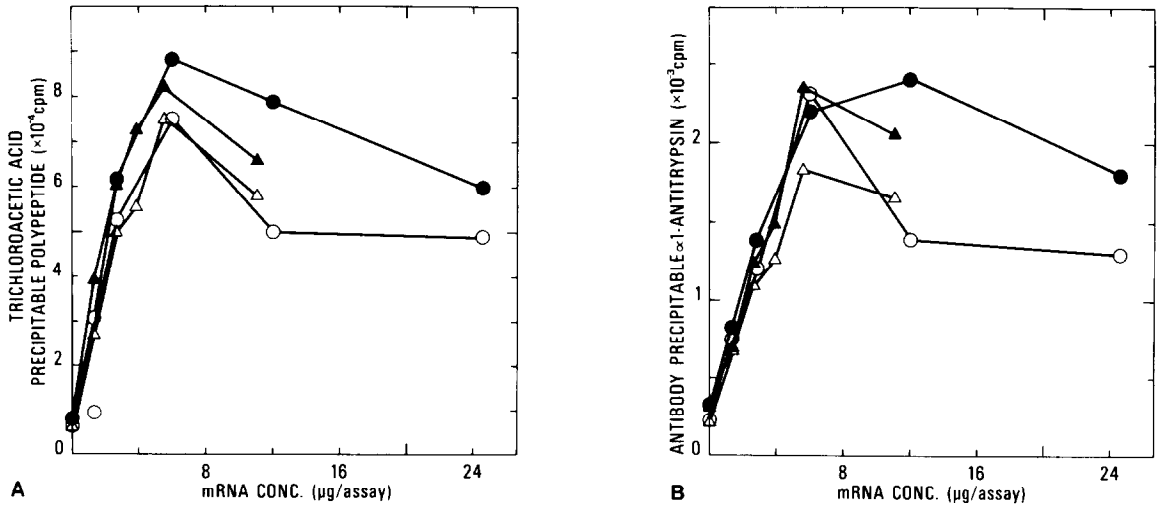

Fig.1. Liver mRNA directed cell-free protein synthesis. mRNA from PiMM phenotype liver in the presence (○), or absence (●) of dog pancreas membranes. mRNA from PiZZ phenotype liver in the presence (△), or absence (▲) of dog pancreas membranes: (A) total incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable polypeptides of both the M and the Z liver mRNA in the presence and absence of dog pancreas membranes; (B) incorporation of [^{35}S]methionine into antibody-precipitable α_1-antitrypsin directed by both the M and Z liver mRNA, again in the presence and absence of dog pancreas membranes.

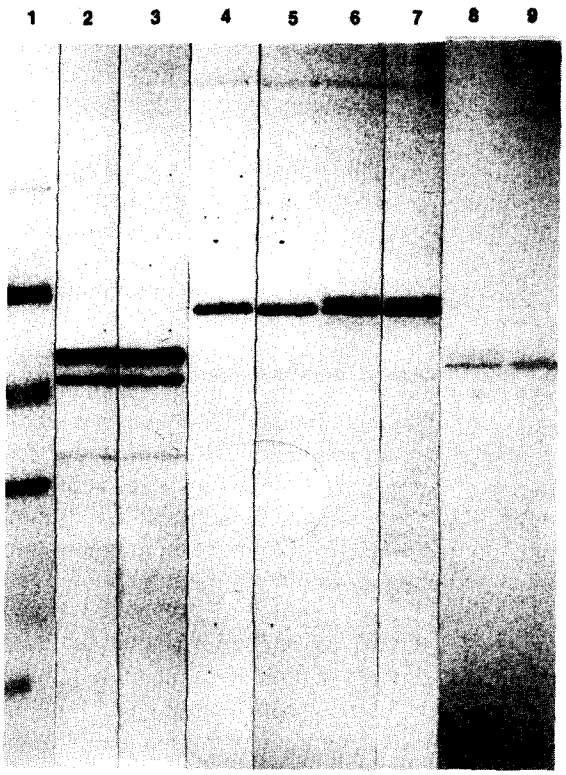

Fig.2. Fluorograph of SDS–polyacrylamide gel showing the synthesis of M and Z α_1-antitrypsin. Antibody precipitates of α_1-antitrypsin synthesised from total liver mRNA in reticulocyte lysate with or without dog pancreas membranes were examined by SDS–polyacrylamide gel electrophoresis. Lane 1: M_r distribution of ^{14}C-labelled markers (5 μl of 5 μCi/ml) (from the top of the gel): phosphorylase B (93000); bovine serum albumin (68000); ovalbumin (45000); carbonic anhydrase (30000); and lysozyme (14200). Lanes 2 and 3: antibody precipitated α_1-antitrypsin synthesised in a reticulocyte lysate (lane 2, PiMM 37000 cpm; lane 3, PiZZ 35000 cpm). Lanes 4 and 5: antibody-precipitated α_1-antitrypsin synthesised in the presence of dog pancreas membranes (lane 4, PiMM 26000 cpm; lane 5, PiZZ 27500 cpm). Lanes 6 and 7: α_1-antitrypsin synthesised in the presence of dog pancreas membranes and treated with proteinase-K prior to antibody precipitation (lane 6, PiMM 21000 cpm; lane 7, PiZZ 21500 cpm). Lanes 8 and 9: antibody-precipitated α_1-antitrypsin synthesised in the presence of dog pancreas membranes and treated with endo-β-*N*-glucosaminidase (lane 8, PiMM 6500 cpm; lane 9, PiZZ 7200 cpm).

presence of membranes were treated with proteinase-K prior to antibody precipitation [13] (fig.2, lanes 6 and 7) this high M_r protein remained. When detergent was used to solubilise the membranes prior to the proteinase-K treatment, the protein was degraded. To confirm that the protein M_r 59000 which was resistant to proteolytic degradation in the absence of detergents, had undergone post-translational modification, antibody-precipitated material was subjected to endo-β-*N*-glucosaminidase digestion. As shown in fig.2 (lanes 8 and 9) this treatment reduced the M_r of α_1-antitrypsin to 47000 and is consistent with the removal of both the core sugar residues and the N-terminal signal sequence.

In this process only 25% of the counts were recovered and a second experiment was performed to exclude the possibility that the single band observed was due to specific proteolytic cleavage. In this second experiment a different membrane preparation was used which appears to give slower post-translational processing. This produced 5 distinct bands: the two of lowest M_r coinciding with the unmodified α_1-antitrypsin polypeptide as from a cell-free system, the remaining 3 being of greater than M_r 49000 and compatible with the sequential addition of each of the 3 carbohydrate sidechains known to be present in α_1-antitrypsin [17] (fig.3). Treatment with endo-β-N-glucosaminidase again reduced all 3 bands to a single band with an M_r 47000, and a 50% yield of radioactivity. Had the product in lanes 8 and 9 of fig.2 been due to specific proteolytic cleavage then multiple bands should still have been observed in lanes 4 and 5 of fig.3.

In PiZZ individuals, α_1-antitrypsin is only partially secreted and a considerable amount accumulates in the endoplasmic reticulum of the hepatocytes. This accumulated protein has the same N-terminal sequence as the plasma form (personal communication, J.-O. Jeppsson), but its carbohydrate sidechains appear to be immature with a high mannose content and no sialic acid [18,19]. This is compatible with the deficiency of the Z protein being due to a partial block in secretion of the polypeptide with only a little of the material being fully glycosylated, and subsequently secreted into the plasma. Our previously published results [6] indicated that the levels of α_1-antitrypsin mRNA as judged by translation in a wheatgerm system are

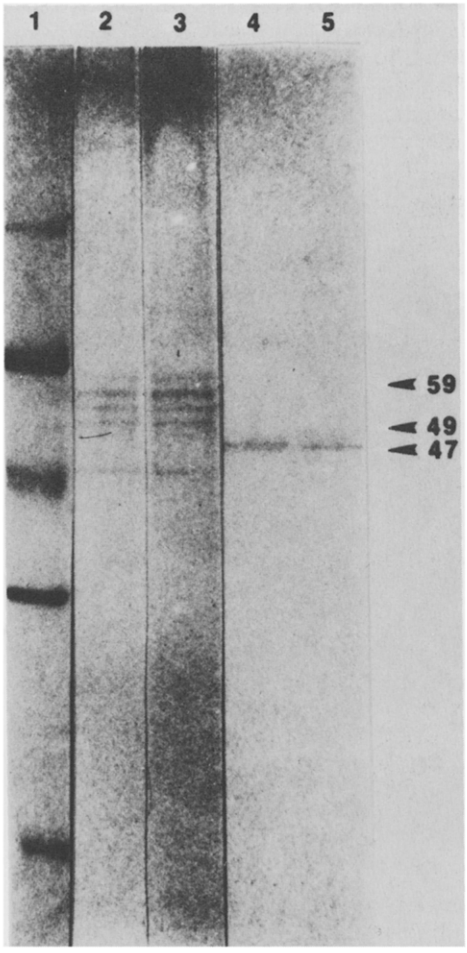

Fig.3. Fluorograph of SDS–polyacrylamide gel showing the post-translational modification of M and Z α_1-antitrypsin. Conditions of synthesis, preparation of mRNA, and antibody precipitation were as described for fig.2. Lane 1: M_r distribution of ^{14}C-labelled markers (as in lane 1, fig.2). Lanes 2 and 3: antibody-precipitated α_1-antitrypsin synthesised in the presence of a second preparation of dog pancreas membranes (lane 2, PiMM 20100 cpm; lane 3, PiZZ 23000 cpm). Lanes 4 and 5: antibody-precipitated α_1-antitrypsin synthesised in the presence of dog pancreas membranes and treated with endo-β-*N*-glucosaminidase (lane 4, PiMM 11000 cpm; lane 5, PiZZ 10400 cpm).

similar for both the PiMM (normal) and PiZZ (abnormal) livers. This therefore implies that the low level of circulating Z α_1-antitrypsin results from defective processing which causes the observed accumulation within the cell [1]. In the series of experiments presented here we have demonstrated that the polypeptide is processed normally by the dog pancreas membranes resulting in the removal of the N-terminal signal sequence and addition of the core sugar residues.

Thus we conclude that the Z mutation in α_1-antitrypsin (Gly $\longrightarrow$ Lys at amino acid 342 [1,20,21]) causes a partial blockage in the processing or transport of the protein subsequent to core glycosylation.

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